

Kentucky
Agricultural Experiment Station
University of Kentucky

**Hemolytic Streptococci of Horses and Other Animals,
and Their Relation to the Streptococci of Man.**

BULLETIN NO. 338

(Research Bulletin)



Lexington, Ky.

May, 1933

EXPERIMENT STATION STAFF

BOARD OF CONTROL

Richard C. Stoll, Chairman, Lexington, Ky.
Joe B. Andrews, Covington, Ky.
R. G. Gordon, Louisville, Ky.
James Park, Lexington, Ky.

Frank L. McVey, President

Thomas P. Cooper, Dean and Director

ADMINISTRATION

T. P. Cooper, Director
D. H. Peak, Business Agent
O. L. Ginocchio, Secretary

AGRONOMY

George Roberts, Head
E. J. Kinney, Associate Agronomist
P. E. Karraker, Asst. Agronomist
J. F. Freeman, Asst. Agronomist
W. D. Valleau, Plant Pathologist
E. N. Fergus, Asst. Agronomist
J. B. Kelley, Agricultural Engineer
E. M. Johnson, Asst. Plant Pathologist
C. E. Bortner, Asst. in Agronomy

ANIMAL HUSBANDRY GROUP

E. S. Good, Chairman
W. S. Anderson, Horses
E. J. Wilford, Swine, Meats
W. J. Harris, Beef Cattle
J. Holmes Martin, Poultry
Fordyce Ely, Dairy Husbandry
W. C. Eskew, Cream Grading
J. W. Nutter, Dairyman
Amanda Harms, Asst. Path. Bact.
G. D. Buckner, Animal Nutrition
W. M. Insko, Jr., Asst. Poultry Husb.
E. A. Baute, Poultry Improvement
Harold Barber, Head Herdsman

ANIMAL PATHOLOGY

W. W. Dimock, Head
Philip R. Edwards, Bacteriologist
F. E. Hull, Asst. Veterinarian
D. J. Healy, Bacteriologist

CHEMISTRY

J. S. McHargue, Head
S. D. Averitt, Chemist
O. M. Shedd, Chemist
W. R. Roy, Asst. Chemist
Robert K. Calfee, Asst. Chemist
David W. Young, Assistant

CREAMERY LICENSE SECTION

J. D. Foster, Inspector in Charge
N. J. Howard, Inspector

ENTOMOLOGY AND BOTANY

W. A. Price, Head
Mary L. Didlake, Asst. Entomologist
H. H. Jewett, Research Asst. Entomologist
Jessie Taylor, Seed Analyst
C. O. Eddy, Assoc. Entomologist
E. C. Vaughn, Seed Production
Encil Deen, Seed Inspector

FARM ECONOMICS

W. D. Nicholls, Head
W. L. Rouse, Farm Management
Z. L. Galloway, Farm Management
Merton Oyler, Rural Life Studies
George B. Byers, Farm Organization
Bruce Poundstone, Farm Management

FEED CONTROL

J. D. Turner, Head
H. D. Spears, Chemist
W. G. Terrell, Inspector
Fred Fitschen, Inspector
L. V. Amburgey, Microscopist

FERTILIZER CONTROL

H. E. Curtis, Head
Harry Allen, Chemist
Lelah Gault, Asst. Chemist
Robert Mathews, Inspector

HOME ECONOMICS

Statie Erikson, Head
Ruth Boyden, Assistant

HORTICULTURE

A. J. Olney, Head
C. S. Waltman, Assistant
E. M. Emmert, Assistant

MARKETS AND RURAL FINANCE

H. B. Price, Head
Dana G. Card, Prices
Clifton J. Bradley, Rural Finance
C. D. Phillips, Markets
Olin M. Farrington, Markets

PUBLIC SERVICE LABORATORY

L. A. Brown, Head
A. L. Meader, Asst. Chemist
James H. Martin, Asst. Chemist
Geo. S. Terry, Bacteriologist
Harvey Cunov, Asst. Bacteriologist
Todd Green, Asst. Bacteriologist

ROBINSON SUBSTATION

(Quicksand, Ky.)

R. W. Jones, Superintendent
G. H. Wiggin, Forester

WESTERN KY. SUBSTATION

(Princeton, Ky.)

S. J. Lowry, Superintendent
L. M. Caldwell, Assistant

BULLETIN NO. 338

(Research Bulletin)

Hemolytic Streptococci of Horses and Other Animals, and Their Relation to the Streptococci of Man.

By W. W. DIMOCK and P. R. EDWARDS

It has been known that streptococcic infections were prevalent among domestic animals almost as long as streptococci have been known as etiologic agents of disease. That the horse is particularly susceptible to infection with streptococci has long been evident. Schutz (1, 2) in 1887 and 1888 established a relationship between respiratory diseases of the horse and streptococcic infection when he isolated streptococci from cases of pneumonia and strangles. The connection of streptococci with diseases of the new-born was proved by Casper (3) in 1897 in his studies of joint ill of foals. In 1901 Ostertag (4) demonstrated streptococcic infection in abortion of mares. Since then many workers have isolated streptococci from horses affected with various pathologic conditions and much work has been done in the study of the organisms involved. No systematic review of the literature is attempted here since Jones (5) and Ogura (6) have published extensive reviews of the literature regarding the role of the streptococci in respiratory infections of the horse, and McFadyean and Edwards (7) and Magnusson (8) have reviewed the literature concerning streptococci in abortion and diseases of the new-born.

In working on the pathology and bacteriology of the genital organs of mares and stallions in relation to sterility and diseases and prenatal infections of the new-born, it was found that diseases due to streptococci were one of the most important

problems confronting the horse-breeding industry. Dimock and Snyder (9) and Dimock and Edwards (10) demonstrated that streptococci are the cause of numerous cases of metritis in mares. Dimock and Edwards (11) further demonstrated that streptococci occur frequently in abortion and diseases of foals and are present in the uterus of the mare following abortion or normal parturition. The writers have published short descriptions of these streptococci in several papers but no systematic study of them. During the past two years the cultural and biochemical characters of these streptococci have been studied in detail. The cultures isolated from genital tracts of mares and from diseased foals have been compared with cultures derived from the respiratory organs of the horse. In addition, strains of streptococci from other species of domestic animals and from various pathologic conditions in humans have been included in the study. The present paper reports the results of this work in detail.

MATERIALS

The streptococci encountered in genital infections and diseases of the new-born in the horse are strikingly similar in their cultural and biochemical reactions. All these organisms are beta hemolytic streptococci which produce only a low acidity in glucose broth and fail to hydrolyze sodium hippurate. The organisms included in the present study are limited to strains having these characters and it should be borne in mind that any statements made in this paper apply only to low-acid-producing, non-sodium-hippurate-hydrolyzing streptococci which are actively hemolytic in fluid mediums.

A number of strains were collected from the os uteri of infected mares and from mares that had recently aborted or given birth to normal foals. These cultures were isolated by the technique originally described by Dimock and Snyder (9). Cultures isolated from fetuses and foals on post-mortem examination, from influenza and infectious rhinitis in foals and adult horses, from wound infection in horses and from strangles or distemper were studied. In addition cultures derived from various

pathologic conditions in cows, hogs, chickens, foxes, guineapigs, and rabbits were compared with the cultures of equine origin. Finally a number of cultures of human origin were compared with cultures isolated from the lower animals. The sources of the cultures are given in table 1. Unless otherwise stated the strains were isolated by the writers. For the convenience of the reader the list is summarized in table 2.

TABLE 1. SOURCE OF CULTURES.

Designation of Cultures	Source
	Cultures of Animal Origin
1-100	Os uteri of mares affected with metritis, and os uteri of mares following abortion or normal parturition.
F1, F13, F25, F26, F27, F28, F29, 10116	Aborted fetuses, septicemia and joint ill of foals.
F14-F24	Nasal discharge, infectious rhinitis of wean- ling foals.
B1, B2, B3	Heart blood, Influenza of Horses.
D1-D4	<i>Str. equi</i> from strangles (adenitis equorum).
D5	<i>Str. equi</i> from strangles. From Dr. F. S. Jones.
D6-D9	<i>Str. equi</i> from strangles. From Maj. R. A. Kelser.
17647	Wound infection of horse.
20790	Septicemia of horse.
18783	Muscle of cow.
25261	Placenta, cow.
25505, 28117	Brain, calf.
	} From Dr. R. Graham.
17207	Acute mastitis, cow. From Dr. F. W. Schofield.
17099	Apparently normal milk. From Dr. W. N. Plastringe.

TABLE 1. SOURCE OF CULTURES (Continued).

17090, 17098	Femur, chicken. Slipped tendon.	} From Dr. R. Graham.
3962, 6554, 18784, 18786, 20735, 21013, 22499, 23155, 30748	Femur, chicken. Slipped tendon.	
10986	Brain, pig.	
22731	Liver, pig.	
25272, 25273, 25274	Fetus, sow.	
12089	Joint, pig. Arthritis.	
31244	Ovaries, sow, following abortion.	
Clark	Natural infection of rabbit. From Dr. J. H. Brown.	
611B	Guinea pig, Lymph-adenitis. From Dr. F. S. Jones.	
GplF, Lumps 4, Lumps 310	Guinea pigs, Lymph-adenitis. From Dr. J. H. Brown.	
3580, 3597	Fox pups, Pneumonia. From Dr. F. W. Schofield.	
Cultures from Human Sources.		
S3, S23, S43, S84	Stock cultures, representative of the four types of Dochez, Avery, and Lancefield (Jour. Exp. Med. 1919, 30, 179). From Dr. R. C. Lancefield.	
H1, H2, H6-H10 H12-H23, H69-H81, H88-H110	Throat cultures, scarlet fever suspects or convalescents, and streptococcic sore throat.	
H3, H4, H5	Mastoiditis. From Dr. E. S. Maxwell.	
H11	Spinal fluid, Meningitis.	
NYV, C203	Stock cultures, <i>Str. scarlatinae</i> . From Dr. A. R. Dochez.	
H24	Scarlet fever.	} From Dr. J. F. Bullard.
H25	Erysipelas.	
H26	Hemorrhagic smallpox.	

TABLE 1. SOURCE OF CULTURES (Continued).

H27-H30	Type cultures, <i>Str. scarlatinae</i> . From Dr. G. F. Dick.	
H31	Erysipelas.	} From Dr. S. A. Koser.
H32-H35	Scarlet fever.	
H36, H37	Lung, following measles.	} From Dr. R. C. Lancefield.
H38	Passage culture NYV.	
H39	Aronson's strain.	
H40, H41	Puerperal sepsis.	
H42, H43	Throat, tonsillitis.	
H44, H45	Sputum, pneumonia.	
H46-H51	Stock cultures, <i>Str. erysipelatis</i> . From Dr. R. Tunncliffe.	
H52	Peritonitis.	} From Dr. L. Thompson.
H53, H54, H55	Mastoiditis.	
H56	Pleuritis.	
H57	Erysipelas.	} From Mr. E. K. Borman.
H58-H63	Scarlet fever.	
H64	Throat culture, milk handler thought to be responsible for epidemic of scarlet fever.	
H65, H66, H67	Scarlet fever.	} From Dr. J. H. Brown.
H68	Broncho-pneumonia.	
H82-H86	Throat swabs for diphtheria.	
	Cultures classified as <i>Str. epidemicus</i> .	
E1-E10, E12, E13	Cultures from mastitis of cows.	} From Dr. W. D. Frost.
E14, E15, E16, E17	Throat cultures from septic sore throat.	

TABLE 1. SOURCE OF CULTURES (Continued).

E21-E25, E27-E31, A, C	Throat cultures.	} From Dr. I. Pilot.
E26	Udder of cow.	
E32, E33, E34	Throat cultures.	From Dr. E. S. Robinson.
E35, E36, E37, E40, E41	Cultures from septic sore throat.	} From Dr. A. W. Williams.
E38, E39	Udders of cows.	
E42	Spleen, fatal case septic sore throat.	} From Dr. J. H. Brown.
E43, E46, E47, E49, E52, E53	Throat cultures, septic sore throat.	
E44	Suppurating ear, septic sore throat.	
E45, E54, E55	Udders of cows.	
E48	Blood, septic sore throat.	
E50	Case of erysipelas during epi- demic of sore throat.	
E51	Infected sinus during epi- demic of sore throat.	} From Dr. F. H. Fraser.
E56, E57	Udders of cows.	
E58-E68	Throat cultures, septic sore throat.	

TABLE 2. SUMMARY OF CULTURES STUDIED.

Source of Culture.	Number
Cultures of Animal Origin.	
Equine, os uteri	100
Equine, aborted fetuses	8
Equine, joint ill and septicemia of foals	11
Equine, infectious rhinitis	11
Equine, influenza	3
Equine, wound infection	1
Equine, septicemia	1
Equine, Str. equi from strangles	9
Bovine, placenta	1
Bovine, septicemia	3
Bovine, milk	2
Chicken, femur	11
Swine, septicemia	2
Swine, abortion	4
Swine, arthritis	1
Rabbit, septicemia	1
Guinea pig, lymph adenitis	4
Fox, pneumonia	2
Cultures from Human Sources.	
Throat cultures, scarlet fever and diphtheria suspects, sore throat, etc.	62
Mastoiditis	6
Scarlet fever, stock cultures	22
Meningitis	1
Erysipelas	9
Pneumonia	9
Smallpox	1
Pleuritis	1
Peritonitis	1
Puerperal sepsis	2
Unknown	1
Cultures Classified as Str. Epidemicus.	
Throat cultures	41
Udders of cows	20
Spleen	1
Blood	1
Erysipelas	1
Infected sinus	1

METHODS

The method of Ayers and Rupp (12) was used in testing for the hydrolysis of sodium hippurate. The organisms were grown for 7 days at 37°C in the following medium:

Beef infusion	1000cc
Peptone (Bacto)	10 grams
Sodium hippurate	10 grams
Reaction adjusted to pH 7.6.	

After incubation, 0.5cc of a 12-percent solution of ferric chloride, containing 2.5cc of concentrated hydrochloric acid per liter, was added to 2cc of the clear supernatant fluid of the culture. An insoluble precipitate indicates the hydrolysis of the hippurate with the formation of benzoic acid.

In determining hemolysin production the ability of the streptococci to dissolve blood cells in a fluid medium was tested. The organisms were cultivated in the following medium:

Beef infusion	1000cc
Peptone (Bacto)	10 grams
Sodium chloride	5 grams
Reaction adjusted to pH 7.6	

The medium was tubed in amounts of 10cc. After sterilization, 20 percent of sterile horse serum was added to the broth. It was seeded with a heavy inoculum of a blood-broth culture of the strains to be tested and incubated at 37°C for 8 hours. At this time a sample was withdrawn and incubation continued. The sample was centrifuged to clarity and placed in small test tubes in amount of 0.5cc, 0.2cc, 0.1cc and 0.5cc. To each tube was added 0.2cc of a 5-percent suspension of washed sheep cells and sufficient saline to make a total volume of 1.0cc. These tubes were incubated at 37°C for one hour and the degree of hemolysis recorded. If hemolysis was not complete in the tube containing 0.05cc of the supernatant fluid of the culture, the culture was tested again after incubation for 24 hours. The cultures that grew rapidly in the serum broth usually showed an early production of hemolysin. These cultures hemolyzed rapidly after 8 hours growth but when tested after 24 hours incubation hemolysis was weak or absent. The cultures which developed more slowly in the broth exhibited

little or no hemolysin after 8 hours incubation, but hemolyzed readily after 24 hours incubation. All the cultures included in the study caused complete hemolysis when 0.05cc was used in the test.

A few cultures of the high-acid-producing, sodium-hippurate-hydrolyzing streptococci from cows were tested for hemolysin production. While these caused more or less hemolysis in blood plate cultures, their hemolytic action in a fluid medium was much weaker than that of the group of streptococci used in this study. This fact has been recorded by Brown (13) who made use of this property in differentiating human and bovine streptococci.

In testing the amount of acid produced from glucose, the cultures were grown in beef infusion peptone broth containing 1 percent glucose and having a reaction of pH 7.6. After 96 hours incubation the hydrogen ion concentration was determined by comparison with standard buffer solutions, using methyl red and brom cresol green as indicators.

In testing ability to utilize fermentable substances the cultures were grown in the following medium:

Beef infusion	900cc
Casein digest	100cc
Andrade's indicator	10cc

The casein digest used was prepared according to the method of Kulp and Rettger (14). The reaction was adjusted to pH 7.4 and the medium tubed and sterilized. The fermentable substances were dissolved in distilled water, sterilized by filtration and added to the broth in sufficient amount to give a concentration of 1 percent. The cultures were incubated at 37°C for 10 days unless fermentation occurred earlier. Fermentation of the substances found useful in differentiating the cultures was prompt and distinct. When fermentation occurred acid production usually was apparent in 24 hours and in only one or two instances were reactions delayed beyond 48 hours.

In examining for capsule production the cultures were grown in beef infusion peptone broth to which had been added 20 percent sterile ascitic fluid. After 18 hours incubation they were examined in moist india ink preparations.

TABLE 3. CHARACTERS OF CULTURES

Designation	Hydrolysis of Sodium Hippurate	Hemolysis	pH in Glucose*	Saline	Mannitol	Lactose	Sorbitol	Trehalose	Capsules	Reduction of Methylene Blue	Greening of Chocolate Agar	Origin
1, 2, 3, 6-35, 37-54, 56-100, F1-F5, F7-F29, B1, B2, B3, 10116, 17674, 20750	-	+	5.0	+	-	+	+	-	+	-	-	Equine
4, 36, 55, F6	-	+	5.0	+	-	-	-	+	-	+	+	
5	-	+	5.0	+	-	+	-	+	-	+	+	
D1-D9	-	+	5.0	+	-	-	-	-	+	-	-	
18783, 25261, 25505, 28117, 17297, 17099, 17090, 17098, 3962, 6554, 18782, 18788, 20735, 21013, 22498, 23155, 30748, 10986, 22731, 23273, 23274, 12698, 31244, 611B, Gp1F, Lumps 4, Lumps 310, Clark, 3380, 3397.	-	+	5.0	+	-	+	+	-	+	-	-	Animals other than Horses
25272	-	+	5.0	+	-	-	-	+	-	+	+	
S3, S23, S84, H5, H7, H10, H11, H15, H16, H18, H20, H21, H24, H25, H27, H28, H30, H33, H34, H35, H36, H38, H42, H44, H45, H49, H51, H53, H54, H55, H57, H58, H59, H60, H61, H62, H63, H64, H75, H85, H87, H88, H89, H93, H96, H98, H99, H109, NYV.	-	+	5.7	+	-	+	-	+	-	-	-	Human

H2, H3, H4, H9, H12, H14, H17, H19, H22, H32, H40, H41, H46, H47, H68, H72, H77, H78, H80, H81, H82, H86, H85, H97, H100, H101, H102, H103, C203, H106, H107, H108, H110, H116, H123, H29, H37, H43, H65, H67	—	+	5.7	+	—	+	—	+	—	+	—	+	—	—	+
H1, H48, H66, H90, H92	—	+	5.6	+	—	+	—	+	—	+	—	+	—	—	+
H11, H31, H83	—	+	5.7	+	—	—	—	—	—	—	—	—	—	—	—
H56, H70, H73, H74	—	+	5.4	+	—	—	—	—	—	—	—	—	—	—	—
H26	—	+	5.6	+	—	—	—	—	—	—	—	—	—	—	—
H69, H79	—	+	5.2	—	—	—	—	—	—	—	—	—	—	—	—
H91	—	+	5.5	—	—	—	—	—	—	—	—	—	—	—	—
H94, H104, H105	—	+	5.2	—	—	—	—	—	—	—	—	—	—	—	—
S43, H39	—	+	6.0	+	+	+	+	+	+	+	+	+	+	+	+
H6	—	+	5.9	+	+	+	+	+	+	+	+	+	+	+	+
H13, H52	—	+	5.6	+	+	+	+	+	+	+	+	+	+	+	+
H8, H50	—	+	5.6	+	—	—	—	—	—	—	—	—	—	—	—
E1-E10, E12-E17, E25, E26	—	+	5.1	+	—	+	+	+	+	+	+	+	+	+	+
E22, E23, E24, E28, E29, E30, E32, E34, E35, E39, E40, E56, E58, E59, E63, E65, E66, E67	—	+	5.7	+	—	+	+	+	+	+	+	+	+	+	+
E27, E31, E33, E42, E43, E44, E45, E48, E49, E50, E52, E53, E57, E60, E61, E62, E68, A, C	—	+	5.4	+	+	+	+	+	+	+	+	+	+	+	+
E36, E37, E38, E41, E47, E51, E54, E55	—	+	5.4	+	—	+	+	+	+	+	+	+	+	+	+
E46, E64	—	+	5.5	+	—	+	+	+	+	+	+	+	+	+	+

*Average.

Reduction of methylene blue is a quantitative test and the results obtained vary widely with the medium used and the amount of methylene blue present. In the present work the cultures were grown in a basic medium of the following composition:

Beef infusion	900cc
Casein digest	100cc
Peptone (Bacto)	5 grams
Reaction adjusted to pH 7.6.	

This medium fostered a luxuriant growth of the organisms, which is necessary if constant results are to be obtained. The cultures were inoculated into tubes of this medium, grown for 18 hours, and 0.1cc of the 18-hour culture was used to inoculate a second tube of the same medium to which had been added sufficient sterile aqueous solution of methylene blue to give a final concentration of .000025 molar. The tubes containing the methylene blue were incubated at 37°C and observed at intervals of 24, 48 and 72 hours. Reduction resulted in a complete decolorization of the medium except for a faint blue ring at the surface of the broth. The cultures either completely decolorized the dye or else did not reduce it at all, as judged by the color present in the tubes.

Action on chocolate agar was determined by the method of Tunnicliff (15). The chocolate agar was prepared by adding 8cc of defibrinated sheep blood to 150cc of melted and cooled plain beef-extract agar, pH 7.0. The mixture was heated to 90°C, cooled to 50°C and poured into plates. The plates were streaked with the cultures to be tested, incubated at 33°C for 72 hours and examined. Any greening of the agar was recorded as a positive result.

TABLE 4. SUMMARY OF CHARACTERS OF CULTURES

Kind	Designation	Number of Cultures	Hydrolysis of Sodium Hippurate	Hemolysis	pH in Glucose Broth*	Saline	Mannitol	Lactose	Sorbitol	Trehalose	Capsules	Reduction of Methylene Blue	Greening of Chocolate Agar
Human		115	—	+	5.7	103+ 6—	110— 5+	104+ 11—	—	+	102— 13+	103— 6+	65— 50+
	Type A	159	—	+	5.0	+	—	—	+	—	+	—	—
Animal	Type B1	5	—	+	5.0	+	—	—	—	+	—	+	+
	Type B2	1	—	+	5.0	+	—	+	—	+	—	+	+
	Str. equi	9	—	+	5.0	+	—	—	—	—	+	—	—
	Laboratory 1	16	—	+	5.1	+	—	+	+	—	+	—	—
Str. epidemicus	Laboratory 2	2	—	+	5.1	+	—	+	+	—	+	—	—
		10	—	+	5.6	+	—	+	—	+	+	—	6— 4+
	Laboratory 3	3	—	+	5.7	+	—	+	—	+	2+ 1—	—	2— 1+
	Laboratory 4	7	—	+	5.5	+	—	+	—	+	3+ 4—	—	4+ 3—
	Laboratory 5	14	—	+	5.5	+	—	+	—	+	10+ 4—	+	13+ 1—
	Laboratory 6	13	—	+	5.5	+	—	+	—	+	12+ 1—	—	8— 15+

*Average.

RESULTS

The results obtained in the study of 354 cultures are given in Table 3. For convenience they are summarized in Table 4. The cultures received as *Str. epidemicus* are divided into groups according to the laboratory from which they were obtained. This was done because the characters of the cultures from the different laboratories were quite different. As can be seen from the tables, all the cultures failed to hydrolyze sodium hippurate and all were actively hemolytic in a fluid medium.

Acid production. No cultures produced a reaction more acid than pH 4.8; that is, all belong to the so-called low-acid-producing group. However different degrees of acid production among the cultures are quite marked. It may be noted that the equine strains, as well as the cultures from other species of animals, produced an average acidity of approximately pH 5.0, while the human strains reached an average acidity of only about pH 5.7. The difference in the degree of acidity produced is not advanced as a differential characteristic of the human and animal cultures, since there is a certain amount of overlapping when individual strains are considered. However, such a marked difference between large groups of cultures indicates a difference in the metabolism of the two groups. Jones (5) noted that equine strains produced a higher titratable acidity in glucose broth than human strains and stated: "Thus acid production appears to serve as a ready means of differentiation between the pathogenic animal and human strains."

Fermentation Reactions. The cultures were tested for their ability to ferment sucrose, lactose, salicin, inulin, rhamnose, raffinose, mannitol, dulcitol, sorbitol, arabinose, xylose, sorbose, trehalose, glycerol, erythritol, inositol, adonitol, populin, glycogen, aesculin, amygdalin, digitalin, arbutin, and melezitose. None of the cultures fermented inulin, rhamnose, raffinose, dulcitol, arabinose, xylose, sorbose, erythritol, inositol, adonitol, populin, aesculin, amygdalin, digitalin, or melezitose. All fermented sucrose and glycogen. The action on glycerol was of no particular differential value and has been reported in a previous paper (Edwards, 16). Arbutin was fermented by an occasional strain of both the human and animal cultures. It was of no

differential value. The action of the cultures on the remaining fermentable substances is given in table 3.

The equine cultures are divided into four types, according to their fermentative reactions. *Str. equi*, represented in table 3 by nine cultures (D1-D9), failed to ferment lactose, sorbitol, and trehalose. In this respect the cultures of *Str. equi* are unique, and differ from all other cultures studied. These strains were isolated from typical cases of strangles and similar cultures have been isolated from no other source.

The remaining equine cultures, with 5 exceptions, fermented sorbitol but failed to attack trehalose. In this respect they differed from the human cultures, which, without exception, produced acid from trehalose but did not ferment sorbitol. The same difference that exists between the human and equine cultures also applies to the cultures derived from animals other than horses. With one exception, the cultures from animals other than horses fermented sorbitol but did not produce acid from trehalose. For this reason it is possible to group the animal strains as a whole and contrast them with cultures of human origin. This has been done in table 4. The animal strains are divided into four types: A, B1, B2, and *Str. equi*. These designations are the ones applied to equine streptococci by Ogura (6). They have been found particularly suited to the cultures studied here and Ogura's designation of the types has been retained. The distinguishing characters of *Str. equi* have already been mentioned. The type A animal strains, which compose 96 percent of the remaining animal cultures, constitute a distinct type. With the exception of certain cultures received as *Str. epidemicus*, which will be considered later, no other cultures were encountered which ferment sorbitol. The sorbitol-fermenting cultures were absolutely constant in all the characters studied. While the human cultures differed more or less in their action on salicin, lactose, and mannitol, and the production of capsules, the type A animal strains all fermented lactose and salicin while none produced acid from mannitol or trehalose. All the type A animal strains produced capsules and none reduced methylene blue or greened chocolate agar.

The type B animal strains differ from the type A cultures

in that they fermented trehalose and failed to ferment sorbitol. All reduced methylene blue and all greened chocolate agar. Type B1 is distinguished from type B2 only by its inability to ferment lactose. While these type B strains resemble the human strains in their action on sorbitol and trehalose there are certain quantitative differences that tend to distinguish them. The percentage of lactose fermenters among the type B strains (16 percent) is much lower than among the human cultures (91 percent). All the type B strains reduced methylene blue, while only 5 percent of the human cultures decolorized the dye. All the type B cultures greened chocolate agar, while less than half the human cultures produced this change. The sources of the type B cultures indicate that they are not human streptococci transferred to animals. Four of the cultures were isolated from the os uteri of mares, one from a foal born dead and one from the ovaries of a sow following abortion. For these reasons the writers regard these cultures as atypical animal strains and not as human strains which have infected animals.

The human cultures varied more or less in most of the characters studied. They were constant, however, in their action on sorbitol and trehalose. All the cultures fermented trehalose and none attacked sorbitol. Six human strains did not form acid from salicin. These were isolated from normal throats of children in institutions where a search was being made for carriers of hemolytic streptococci. None of the human cultures from definite pathological conditions failed to ferment salicin. Four of the salicin-negative strains also failed to ferment lactose, belonging to the *Str. subacidus* group of Holman (17). The remaining two fermented lactose and would fall into Holman's *Str. anginosus*. It is significant that these, as well as the other human strains, fermented trehalose.

The cultures which were sent to us as being *Str. epidemicus* are divisible into two main groups. The first group, composed of 16 cultures from laboratory 1, and 2 from laboratory 2, are identical with the type A animal strains. These cultures fermented sorbitol and failed to ferment trehalose. They were the only cultures studied except the type A animal strains to have these characters. Like the animal strains all produced capsules.

Of the 16 cultures from laboratory 1, twelve were isolated from cases of mastitis in cows. The remaining four were said to be of human origin. Three of these had been received from another laboratory. Duplicates of these three were obtained from the laboratory that originally distributed them. The duplicates were found to resemble human cultures and fermented trehalose but did not attack sorbitol. No duplicate of the fourth culture said to be of human origin could be obtained. The two cultures from laboratory 2 which resembled the animal strains had come originally from laboratory 6. One was said to have been isolated from a human throat, the other from the udder of a cow thought to be responsible for an epidemic of septic sore throat. Duplicates of these cultures were received from laboratory 6. Both resembled the human cultures in their action on sorbitol and trehalose.

The second group of cultures of *Str. epidemicus* is made up of ten received from laboratory 2 and all those from laboratories 3, 4, 5, and 6. All resembled the human type. They fermented lactose, salicin and trehalose and failed to attack mannitol and sorbitol. None of these cultures reduced methylene blue. While *Str. epidemicus* is classified as a capsule producer, It is well known that capsules may not be found on old laboratory strains. Capsules were demonstrated on the majority of the cultures studied; some of them, however, showed no evidence of capsule production in the routine tests. Several of the cultures which showed no capsules were subjected to animal passage. After passage thru animals, capsules were easily demonstrated on these strains.

DISCUSSION

The literature on the differentiation of human and animal streptococci is surprisingly meager. Practically all the studies of animal streptococci can be divided into two groups; first, efforts to distinguish *Str. equi* of strangles from other hemolytic streptococci of the horse, particularly those present in influenza; and second, attempts to distinguish bovine streptococci found in market milk and cases of mastitis from human streptococci, particularly those present in milk-born septic sore throat.

As stated previously, the literature on the differentiation of *Str. equi* and other streptococci occurring on the membranes of the respiratory tract of the horse has been summarized fully by Jones (5) and Ogura (6). The more important contributions to the knowledge of this subject are those of Holth (18), Adersen (19), and Ogura (6). These workers have studied more than 250 cultures of *Str. equi* and found them absolutely constant in their biological characters. They found that the organisms uniformly ferment salicin, fail to ferment lactose, sorbitol or trehalose, and produce distinct capsules. No other equine streptococci possess these characters. Further, it was clearly demonstrated by Zlatogoroff, Kandyba and Sadowsky (20) that *Str. equi*, after long periods of cultivation on artificial media and many transfers, is capable of producing strangles when placed on the nasal mucous membranes of susceptible horses. Other types of equine streptococci were not capable of producing the disease. In addition Ogura (21) and Zlatogoroff, Kandyba and Sadowsky (22) have successfully immunized horses against strangles by the use of pure cultures of *Str. equi*. The fact that *Str. equi* differs in biology, pathogenicity, and immunizing properties from other species of streptococci justifies its recognition as a definite bacteriological entity and its inclusion in classifications as a distinct species. After the recognition of *Str. equi* as a distinct organism two questions present themselves.

First, is *Str. equi* found in conditions other than strangles and does it occur in animals other than horses and in humans? In answer it may be said that *Str. equi* probably is constantly present in cases of strangles. While the experience of the writers with this condition is limited, Holth (18), Adersen (19) and Ogura (6) in careful studies of a large number of cases found this type of streptococcus in all. Ogura (6) showed that other hemolytic streptococci may be associated with *Str. equi* in strangles. This condition is to be expected. Webster and Clow (23) demonstrated that in humans the common cold is accompanied by a great increase in the hemolytic streptococcic flora of the throat and their invasion of the nasal passages. Jones (5) demonstrated that hemolytic streptococci

are normal inhabitants of the pharynx of the horse. However, in normal horses which have not been exposed to respiratory diseases or shipped from point to point hemolytic streptococci are not present on the nasal mucous membranes. Jones (5) demonstrated further that in cases of influenza the mucus membranes of the nose and eye are found to harbor hemolytic streptococci. The writers have found hemolytic streptococci constantly present on the mucous membranes of foals affected with infectious rhinitis. It is probable that any disease affecting the respiratory system of the horse results in a great increase of the streptococcal flora of the nasal mucous membranes. It is not surprising, therefore, that streptococci other than *Str. equi* are isolated from strangles. This is especially true where the material taken for examination is obtained from the nasal mucous membranes and not from abscessed lymph glands.

In regard to the presence of *Str. equi* in conditions other than strangles, it probably does occur rarely in other pathologic conditions in horses. In the examination of 135 cultures of hemolytic streptococci from pathologic conditions other than strangles in horses, 31 cultures from animals other than horses, and 115 cultures of human origin, the writers never encountered *Str. equi*. However, Ogura (6) reported the isolation of 12 strains of *Str. equi* from abscesses and lymphangitis in horses. He remarked that these cultures were from horses not exhibiting typical symptoms of strangles. How many of these cases should have been classified as atypical strangles is problematical. Ogura's findings are in agreement with the observation of Zlatogoroff, Kandyba and Sadowsky (22) that *Str. equi* when inoculated under the skin or into the vaginal mucous membranes does not exhibit its specific pathogenic character, but acts as an ordinary pyogenic streptococcus.

While the occasional occurrence of *Str. equi* in horses not exhibiting typical symptoms of strangles is recognized, there is no question but that many organisms classified as *Str. equi* were not in reality members of this species. Jones (5) classified 4 cultures of streptococci from cases of influenza as *Str. equi*. These cultures were placed in this species because of their inability to ferment lactose. It will be noted that the majority of

the type B animal strains studied by the writers did not ferment lactose. While the type A animal strains all ferment lactose, lactose-negative type B strains are met with frequently in animal streptococci, both in horses and other animals. One is not justified in calling a hemolytic streptococcus *Str. equi* because it does not ferment lactose. Before such a culture is classified as *Str. equi* it should be shown that the culture is a low-acid-producing, non-sodium-hippurate-hydrolyzing streptococcus which ferments neither sorbitol nor trehalose. One frequently encounters papers in which non-lactose-fermenting streptococci of human origin are called *Str. equi*. There is no authentic record of the isolation of *Str. equi* from human sources and it probably does not occur in human infections. It is also doubtful if this species occurs in animals other than horses.

Most of the comparative studies of human and animal strains have been efforts to distinguish streptococci in market milk and bovine mastitis from human streptococci. Ayers (24), Ayers, Johnson and Davis (25), and Avery and Cullen (26) demonstrated that hemolytic streptococci from udders of cows and from dairy products produced a higher acidity in glucose broth than human cultures. Davis (27) found that hemolytic streptococci found in pasteurized and certified milk differed from human cultures in their higher resistance to heat, lack of pathogenicity for rabbits and their rapid growth and acid production in milk. Brown (13) found that bovine cultures produced a higher acidity in glucose broth and were less actively hemolytic in fluid mediums than human strains. Jones (28) described two types of streptococci from market milk; one produced high acidity in glucose broth and was not extremely hemolytic; the other produced less acid in glucose broth, was more actively hemolytic, and failed to ferment salicin. Ayers and Rupp (12) demonstrated that hemolytic streptococci of bovine origin hydrolyzed sodium hippurate whereas human cultures did not. Avery (29) demonstrated that the high-acid-producing, sodium-hippurate-hydrolyzing cultures of bovine origin tolerated concentrations of methylene blue which inhibited the human strains. Brown, Frost and Shaw (30) concluded

that streptococci in milk which produced only a low acidity in glucose broth, failed to hydrolyze sodium hippurate, were pathogenic for mice, produced capsules, and fermented lactose and salicin while failing to ferment mannitol were of human origin. They classified such cultures as *Str. epidemicus*. Frost, Gumm and Thomas (31) and Haynes (32) studied a large number of cultures isolated from certified milk and found that they differed in one or more particulars from human streptococci.

After a review of these references the reader is led to believe that bovine streptococci can be differentiated from human streptococci without great difficulty. While it is true that the majority of bovine strains can be differentiated from human cultures, there remains a group of streptococci which infect cows and other species of domestic animals, which possess all the characters mentioned above as attributes of human strains. After a careful study of a number of streptococci derived from cows, horses and guinea pigs, Smith (33) could not differentiate them from human streptococci. Seelemann and Hadenfeldt (34) isolated streptococci from the uterus and udders of cows which could not be distinguished from human streptococci. They were led to conclude that *Str. pyogenes* of bovine origin was identical with *Str. pyogenes* of human origin. Hergesell (35) in studying the strains isolated by Seelemann and Hadenfeldt, and Diernhofer (36) came to the same conclusion. Minett and Stableforth (37) isolated a number of cultures from cows which they could not distinguish from streptococci of human origin. It may be seen, then, that there is a great group of streptococci of animal origin which have not been successfully differentiated from the human strains. It is with this group that the present writers have worked. The great majority of this group have been successfully differentiated from the human strains.

By reference to table 3 it may be seen that of 135 cultures of equine origin (exclusive of *Str. equi*), 130 fell into a single well-defined type (Type A). This group is made up of cultures from genital infections of mares, aborted fetuses, foals, and respiratory disturbances such as influenza, pneumonia and infectious rhinitis. It is of interest that the cultures from geni-

tal infections should be indistinguishable from cultures obtained from diseases of the respiratory tract. It has been stated earlier that any respiratory disease leads to invasion of the mucous membranes of the throat and nose by hemolytic streptococci. The same condition prevails in the genital tract. It has been demonstrated by Dimock and Snyder (9) that hemolytic streptococci are present on the lips of the vulva of normal mares. Dimock and Edwards (11) have shown that hemolytic streptococci rapidly invade the uterus following abortion or normal parturition.

By further reference to table 3 it may be seen that 30 of 31 cultures from other species of domestic and laboratory animals belong to the same type as the 130 equine cultures. All these cultures possess the characters which, in the past, have been attributed to streptococci of human origin. It has been demonstrated, however, that these cultures can be differentiated from human strains by their action on sorbitol and trehalose. All of this group of streptococci ferment sorbitol and fail to ferment trehalose. The human cultures, on the contrary, without exception produce acid from trehalose but do not attack sorbitol. It is of great interest that this type of streptococcus, composing 96 percent of the animal strains, should be so widely distributed thruout the various species of animals. It becomes apparent that an organism of this type should not be spoken of as an equine streptococcus or a bovine streptococcus, depending on the species from which it is isolated. It is in every sense an animal streptococcus and its capacity to produce disease is not limited to one species of animal.

After the differentiation of the type A animal strains, 6 cultures remain which act upon soribtol and trehalose as do streptococci of human origin. These have been called type B animal strains. Like the type A animal strains their presence is not limited to one species of animal. Of the 6 studied here, 5 were derived from horses and 1 from a hog. It is probable that these strains also occur in other species. The proportion of strains of type B to type A is small and a sufficient number of cultures has not been examined to determine their distribution. While these cultures have not been clearly differentiated

from human strains, reasons have been given previously for regarding them as animal cultures. In general the pathogenicity of this type for the laboratory animals is low. It is sometimes found in organs and tissues showing no pathologic changes. It also may be found associated with type A animal streptococci or *Str. equi*. Its importance as a primary agent in the production of disease is probably slight.

All the 115 human cultures studied possess the properties that have been considered characteristic of these strains. All are actively hemolytic, low-acid-producing, non-sodium-hippurate-hydrolyzing streptococci. However, as has been stated, these characters do not distinguish them from the group of animal streptococci being studied. The cultures of human origin differ in all the remaining characters studied except their action on sorbitol and trehalose. In their action on these two substances the cultures are constant. From the results obtained in this study it seems justifiable to conclude that sorbitol-fermenting streptococci are rarely if ever the cause of disease in humans. Fraser (38) stated recently that in testing over 300 strains of human origin, all were found to ferment trehalose, but none fermented sorbitol.

While the animal strains as yet are imperfectly distinguished from the human type, on account of the similarity of the type B cultures to the human type, it is possible to separate definitely the great majority of the hitherto undifferentiated animal cultures by the methods employed in this study. It seems probable that a final separation of the human and animal streptococci eventually will be effected.

The observations of Tunnicliff (15) that streptococci of erysipelas and septic throat greened chocolate agar while streptococci of scarlet fever left this medium unchanged have not been confirmed. It was found that certain strains from erysipelas and septic sore throat failed to produce greening, while some scarlet fever streptococci were active in producing the color.

STREPTOCOCCI CLASSIFIED AS *STR. EPIDEMICUS*.

In the work reported above it was demonstrated that there is a large group of hemolytic streptococci of animal origin which

possess the characters that have been attributed to human strains. It was shown further that these strains occur in cows, as well as other species of domestic animals. This fact is extremely important since it modifies all the work that has been done on the differentiation of human and bovine streptococci. A low-acid-producing, non-sodium-hippurate-hydrolyzing, actively hemolytic streptococcus recovered from dairy products or from the udder of a cow, can no longer be considered as being necessarily of human origin. It may be an animal streptococcus belonging to the group under consideration.

The fact that the type A animal strains ferment lactose, sucrose and salicin, fail to ferment mannitol, produce distinct capsules, and are pathogenic for laboratory animals, leads to further confusion, since these are the characters attributed by most workers, notably Brown, Frost, and Shaw (30), to *Str. epidemicus*. Not only do the type A animal strains produce distinct capsules but under proper conditions of culture they produce large, spreading, mucoid colonies. Thus they fulfill the requirements laid down by Pilot, Hallman and Davis (39) for classification as *Str. epidemicus*. Further evidence of their similarity to this species is the finding (see table 4) of 18 cultures of sorbitol-fermenting streptococci indistinguishable from the animal strains classified as *Str. epidemicus*. Further, Hadley and Frost (40) have classified cultures of equine origin obtained from the writers as *Str. epidemicus*.

It is the opinion of the writers that sorbitol-fermenting streptococci are strictly of animal origin and that they do not cause epidemics of septic sore throat. In support of this opinion the following facts may be adduced: Of the 16 cultures from laboratory 1, which were identical with the type A animal strains, twelve were isolated from cows. The remaining four strains were said to be of human origin. Duplicates of 3 of these four cultures were obtained from the laboratory originally distributing them. These cultures resembled the human strains. No duplicates of the fourth culture could be obtained.

The two cultures from laboratory 2 which resembled the type A animal strains, were obtained originally from laboratory 6. Duplicates of these two cultures were obtained directly

from laboratory 6. The duplicates were members of the human type. It seems, therefore, that these inconsistencies may be explained by assuming that these cultures had been mislabelled or that an admixture of types had taken place. These sorbitol-fermenting streptococci from laboratories 1 and 2 certainly cannot be considered as authentic cultures of septic sore throat strains.

Recently a number of publications have appeared in which streptococci apparently identical with *Str. epidemicus* have been found in cases of bovine mastitis which had no connection with human disease. Hadley (41) recorded the isolation of organisms classified as *Str. epidemicus* from 29 cows in 10 herds. He stated: "It is significant that the milk from only two of these herds was associated with septic sore throat." Minett and Stableforth (37) isolated streptococci which they could not distinguish from *Str. epidemicus* from 13 cows in 10 herds. There was no record of septic sore throat among the milkers or consumers of the milk. Seelemann and Hadenfeldt (34, 42) isolated streptococci which they considered identical with *Str. epidemicus* from the uterine discharge and udders of cows. There was no record of septic sore throat being connected with the disease. Steenblock (43) isolated streptococci which were classified as *Str. epidemicus* from 3 cows in 2 herds. No ill effects followed the use of the milk.

It seems probable, when streptococci conforming to previous descriptions of *Str. epidemicus* are present in milk without producing ill effects in the consumers, that these streptococci may be, in reality, type A animal streptococci. Dr. Minett (44) has since stated that the streptococci which Minett and Stableforth (37) described fermented sorbitol, indicating that they were of animal origin.

Further work should be done on the production of capsules by the hemolytic streptococci. Capsule production in this group formerly was considered to be a property possessed exclusively by *Str. epidemicus*. This view is no longer tenable. Tunnicliff (45) showed that capsules were produced by the smooth forms of scarlet fever and erysipelas streptococci. Brown and Kindwall (46) found that streptococci which ordinarily did not pro-

duce capsules could be made to produce them if grown on blood-milk agar. The production of capsules was accompanied by the formation of large, mucoid colonies.

The writers have observed capsules on the type A animal cultures and have confirmed the observations of Brown and Kindwall. Fraser and Plummer (47) found capsules no more constantly on septic sore throat streptococci than on strains from other pathologic conditions in humans. From these observations it may be deduced that capsule production is a much more common property of the hemolytic streptococci than has been recognized.

That human streptococci are found in mastitis is indicated by the fact that 10 cultures classified as *Str. epidemicus*, and belonging to the human type, were isolated from the udders of cows thought to be responsible for epidemics. Jones and Little (48) demonstrated that scarlet fever streptococci may become established in the udder. Golledge (49) demonstrated that acute streptococcic septicemia in the human may result from contact with cows carrying human streptococci.

In conclusion it may be said that the sorbitol-fermenting streptococci of animal origin (type A) occur in mastitis of cows but they probably do not cause epidemic sore throat, and that they are rarely, if ever, present in human disease. Human streptococci, on the contrary, may become established in the udder of the cow, and produce pathologic changes. The infection may then be transferred back to man.

SUMMARY AND CONCLUSIONS

1. There is a large group of low-acid-producing, non-sodium-hippurate-hydrolyzing, hemolytic streptococci which infect horses and other species of lower animals.

2. *Streptococcus equi* is a specific bacteriological entity which can be distinguished from other hemolytic streptococci studied by its inability to ferment lactose, sorbitol, or trehalose.

3. Cultures from influenza, pneumonia and infectious rhinitis of horses were found identical with cultures from metritis of mares and joint ill and septicemia of foals.

4. Cultures from cows, hogs, chickens, foxes, rabbits and guinea pigs were indistinguishable from the streptococci from horses.

5. The animal cultures, other than *Str. equi*, are divisible into three types according to their reactions on lactose, sorbitol and trehalose. Ninety-six percent of the animal strains ferment sorbitol but do not ferment trehalose.

6. Human cultures, without exception, were found to ferment trehalose but did not attack sorbitol.

7. The sorbitol-fermenting cultures often are mistaken for *Str. epidemicus* since they produce distinct capsules.

8. Sorbitol-fermenting hemolytic streptococci probably do not cause septic sore throat and are rarely, if ever, present in human disease.

REFERENCES

1. Schutz. 1887. Virchow's Arch. Path. Anat. u. Physiol., 107, 356.
2. Schutz. 1888. Arch. f. wiss. u. prakt. Tierheilk., 14, 172.
3. Casper. 1897. Deut. tierärztl. Wehnschr., 5, 159.
4. Ostertag. 1901. Monatsch. prakt. Tierheilk., 12, 392.
5. Jones, F. S. 1919. Jour. Expt. Med., 30, 159.
6. Ogura, K. 1929. Jour. Jap. Soc. Vet. Sci., 8, 174.
7. McFadyean, J. and Edwards, J. T. 1919. Jour. Comp. Path. and Ther., 32, 42.
8. Magnusson, H. 1919. Jour. Comp. Path. and Ther. 32, 143.
9. Dimock, W. W. and Snyder, E. M. 1923. Jour. Amer. Vet. Med. Assoc., 64, 288.
10. Dimock, W. W. and Edwards, P. R. 1928. Ky. Agr. Exp. Sta. Bul. 286
11. Dimock, W. W. and Edwards, P. R. 1932. Ky. Agr. Exp. Sta. Bul. 333.
12. Ayers, S. H. and Rupp, P. 1922. Jour. Infect. Dis., 30, 388.
13. Brown, J. H. 1920. Jour. Exper. Med., 31, 35.
14. Kulp, W. L. and Rettger, L. F. 1924. Jour. Bact., 9, 357.
15. Tunncliffe, R. 1930. Jour. Amer. Med. Assoc., 94, 1213.
16. Edwards, P. R. 1932. Jour. Bact., 23, 259.
17. Holman, W. L. 1916. Jour. Med. Research, 34, 377.
18. Holth, H. Quoted by C. O. Jensen in Handb. d. Serumtherap. u. Serumdiag. Klimmer u. Wolf-Eisner. 1911, 226.
19. Adersen, V. 1915. Centralbl. f. Bakt., Abt I, Orig., 76, 111.
20. Zlatogoroff, S., Kandyba, L., and Sadowsky, J. 1930. Centralbl. f. Bakt., Abt. I. Orig., 118, 354.
21. Ogura, K. 1930. Jour. Jap. Soc. Vet. Sci., 9, 31.
22. Zlatogoroff, S., Kandyba, L., and Sadowsky, J. 1930. Centralbl. f. Bakt., Abt. I, Orig., 118, 354.
23. Webster, L. T. and Clow, A. D. 1932. Jour. Exper. Med., 55, 445.
24. Ayers, S. H. 1916. Jour. Bact., 1, 84.
25. Ayers, S. H., Johnson, Jr., W. T. and Davis, B. J. 1918. Jour. Infect. Dis., 23, 290.
26. Avery, O. T. and Cullen, G. E. 1919. Jour. Exper. Med., 29, 215.
27. Davis, D. J. 1916. Jour. Infect. Dis., 19, 236.
28. Jones, F. S. 1920. Jour. Exper. Med., 31, 347.
29. Avery, R. C. 1929. Jour. Exper. Med., 50, 463.
30. Brown, J. H., Frost, W. D., and Shaw, M. 1926. Jour. Infect. Dis. 38, 381.
31. Frost, W. D., Gumm, M. and Thomas, F. B. 1927. Jour. Infect. Dis., 40, 698.
32. Haynes, E. 1929. Jour. Infect. Dis., 45, 316.
33. Smith, J. 1929. Jour. Path. and Bact., 32, 401.

34. Seelemann, M. and Hadenfeldt, A. 1930. *Centralbl. f. Bakt., Abt. I, Orig.*, 118, 331.
35. Hergesell, W. 1931. *Arch. f. wiss. u. prakt. Tierheilk.*, 63, 543.
36. Diernhofer, 1930. *Arch. f. wiss. u. prakt. Tierheilk.*, 61, 181.
37. Minett, F. C. and Stableforth, A. W. 1931. *Jour. Comp. Path. and Ther.*, 44, 114.
38. Fraser, F. H. 1933. Personal communication.
39. Pilot, I., Hallman, B., and Davis, D. J. 1930. *Jour. Amer. Med. Assoc.*, 95, 264.
40. Hadley, F. B. and Frost, W. D. 1933. *Cornell Vet.*, 23, 40.
41. Hadley, F. B. 1931. *No. Amer. Vet.* 12, No. 9, 29.
42. Seelemann, M. and Hadenfeldt, A. 1932. *Centralbl. f. Bakt., Abt. I, Orig.*, 126, 231.
43. Steenblock, J. 1932. *Tierärztl. Rundschau*, 38, 561.
44. Minett, F. C. 1933. Personal communication.
45. Tunncliffe, R. 1931. *Jour. Infect. Dis.*, 48, 511.
46. Brown, J. H. and Kindwall, J. A. 1928. *Proc. Amer. Assoc. Med. Milk Comms.*, 109
47. Fraser, F. H. and Plummer, H. 1931. *Trans. Roy. Soc. Canada, Sec. 5, Biol. Sci.*, 25, 111.
48. Jones, F. S. and Little, R. B. 1928. *Jour. Exper. Med.*, 47, 945, 957, 965.
49. Gollodge, S. V. 1932. *Vet. Rec.*, 44, 1499.

